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(54) **AUTO-CALIBRATED SCANNING-ANGLE PRISM-TYPE TOTAL INTERNAL REFLECTION MICROSCOPY FOR NANOMETER-PRECISION AXIAL POSITION DETERMINATION AND OPTIONAL VARIABLE-ILLUMINATION-DEPTH PSEUDO TOTAL INTERNAL REFLECTION MICROSCOPY**

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See application file for complete search history.

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ABSTRACT

A method, apparatus, and system for improved VA-TIRFM microscopy. The method comprises automatically controlled calibration of one or more laser sources by precise control of presentation of each laser relative a sample for small incremental changes of incident angle over a range of critical TIR angles. The calibration then allows precise scanning of the sample for any of those calibrated angles for higher and more accurate resolution, and better reconstruction of the scans for super resolution reconstruction of the sample. Optionally the system can be controlled for incident angles of the excitation laser at sub-critical angles for pseudo TIRFM. Optionally both above-critical angle and sub critical angle measurements can be accomplished with the same system.

50 Claims, 17 Drawing Sheets

